Concentrations of Interleukin- 1β , Interleukin-6, Interleukin-8 and TNF- α in Cerebrospinal Fluid from Children with Septic or Aseptic Meningitis

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Summary: Cytokines at an inflammatory site may be a better indicator of the clinical severity of an infectious disease than the serum levels of the cytokines. Concentrations of interleukin- 1β (IL- 1β) in paired samples of cerebrospinal fluid (CSF) from 10 rabbits with experimental bacterial meningitis caused by *H. influenzae* type b, were measured, and compared to the concentrations of four cytokines; IL- 1β , interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) in CSF samples from 45 children with or without meningitis. The IL- 1β concentrations in the CSF from rabbits with experimental meningitis were significantly higher than the concentrations in control animals without meningitis ($p < 0.001$). The mean CSF concentrations of IL-8 from meningitic children were significantly higher than in the control group without meningitis ($p < 0.005$). TNF- α was only detected in septic meningitis. Assays of IL-6, however, were not significantly different in the septic meningitis group, the aseptic meningitis group and the non-meningitis group. These data indicate a possible role of IL- 1β , IL-8 and TNF- α as mediators in the meningeal inflammatory process in patients with meningitis and TNF- α , in particular, may play a role in the pathogenesis of septic meningitis.

Key words: interleukin- 1β (IL- 1β) — interleukin-6 (IL-6) — interleukin-8 (IL-8) — tumor necrosis factor (TNF- α) — enzyme-linked immunosorbent assay (ELISA) — meningitis

Introduction

Meningitis is one of the most critical diseases in children. Septic meningitis is especially serious in infants and young children, with a mortality of 2 to 5% and neurological sequelae in 20% or more of the survivors (Klein et al. 1986; Magmoud, and McCracken, 1989). In spite of the new, more active antimicrobial drugs for the treatment of bacterial meningitis, the outcome has not yet improved. Fur-

ther understanding of the pathophysiologic mechanisms of central nervous system injury during bacterial meningitis is needed to provide new therapeutic directions.

The development of immune system cells and the body's response to infection or trauma are controlled by a group of low molecular weight proteins called cytokines. Most cytokines have multiple cellular sources and targets, and share functions to modulate the levels and ef-

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fects of each other. Recent studies have emphasized the importance of cytokines and polypeptide growth factors in controlling the intensity of inflammation, and the tendency for scarring. Macrophages are a uniquely rich source of these factors and they release large amounts of IL-1, IL-6, IL-8 and TNF when activated. The complexities of this integrated network of cytokines are just being understood. For example, both TNF and IL-1 activate inflammatory cells and induce them to synthesize other cytokines, including IL-8, a chemotactic factor for neutrophils and lymphocytes (Cotran and Pober, 1989). IL-1 β is one of two distantly related forms of IL-1; the other form being IL-1 α . Both forms of IL-1, originally known as Lymphocyte Activating Factor (LAF) (Oppenheim et al. 1986), bind to the same cell surface receptor with similar binding kinetics. IL-6 works synergistically with IL-1 to promote B-cell growth, and it has a wide variety of biological activities including mediation of fever and induction of the acute phase response of inflammation (Dinarello, 1988). IL-8 is a potent neutrophil chemotaxin and it promotes up-regulation of certain membrane adhesion receptors in neutrophils. Intradermal or intraperitoneal injection of IL-8 is known to induce rapid local neutrophil accumulation (Detmers et al. 1990). There are two forms of TNF, TNF- α (cachectin) and TNF- β (lymphotoxin), which differ in structure but compete for the same cellular receptor and have virtually identical actions. TNF- α has a role in inflammation and microbial immunity as well as an antitumor action. The bioactivities of TNF- α are activation of neutrophils, induction of the secretion of IL-1 and protection against certain viruses in vitro. TNF- α has also been suspected to serve as a pathogenic mediator in septic shock (Tracey et al. 1987). In experimental meningitis models, an endotoxin of

Haemophilus influenzae type b stimulated the release of mediators for meningeal inflammation, such as IL-1 β and TNF- α . Additionally, intracisternal inoculation of rabbits with IL-1 β induced cerebrospinal fluid (CSF) pleocytosis and blood-brain barrier injury similar to that induced by endotoxin or live bacteria (Mustafa et al. 1989). IL-1 β in the CSF of rabbits with experimental meningitis was initially determined to confirm the existence of cytokines in inflamed CSF. Then the activities of four cytokines, IL-1 β , IL-6, IL-8 and TNF- α , in human CSF were analyzed. These cytokines are considered to be particularly as important as inflammatory mediators against infections. Little data on the relationships between cytokines in the CSF from meningitis patients have been reported (McCracken et al. 1986). This study was undertaken to compare the levels of four cytokines in patients with septic or aseptic meningitis to those in a non-meningitis group.

Materials and Methods

Production of experimental meningitis

A model, originally described by Dacey et al. (1985) and Sande et al. (1974), was used in a modified form. Ten New Zealand white male rabbits (2-3 kg) were anesthetized with 25 mg/kg pentobarbital and immobilized in a stereotactic frame. A spinal needle (3.5 inches, 20 gauge) was introduced into the cisterna magna. Meningitis was induced by intracisternal injection of 0.2 ml of an inoculum of 10^8 - 10^9 colony forming units (cfu) of *H. influenzae* type b, β -lactamase (+) that was originally isolated from the CSF of an infant with meningitis. CSF samples were taken from the rabbits just before inoculation to be used as controls (0h), and at 18 h and 24 h after inoculation with the organism, to determine the IL-1 β concentrations and make cultures.

Bacterial counts in CSF were enumerated to affirm meningitis. Experimental meningitis was diagnosed by clinical symptoms (lethargy, convulsions) and CSF findings (turbid appearance, increased cell counts and positive bacterial cultures).

Patients

Forty-five patients, 24 boys and 21 girls, were studied. Their mean age was 57 months (range; 1~264 months of age). The numbers of patients with septic meningitis, aseptic meningitis and other diseases without meningitis (febrile disease, febrile convulsions, epilepsy and colitis) were 9, 17 and 19, respectively. All patients with septic meningitis were treated by intravenous injections of appropriate and conventional antibacterial drugs.

Assays of cytokines

All cerebrospinal fluid samples (0.5~1.0 ml) were taken at room temperature (18.0~25.0°C) and immediately stored at -20.0~-80.0°C until assayed.

1. IL-1 β in rabbit CSF

Concentrations of CSF IL-1 β in experimental rabbit models were measured by enzyme-linked immunosorbent assay (ELISA) as follows: goat monoclonal antibodies to rabbit IL-1 β were used as the coating antibody (courtesy of Prof. H. Yoshinaga, Kumamoto University, Japan). IL-1 β was then detected by the sequential addition of biotinylated goat monoclonal antibody against rabbit immunoglobulin. O-phenylenediamine dihydrochloride was used as the chromogen. The ELISA plate was read at 492 nm (Titertek Multiskan PLUS, Fliw Laboratories, Irving, UK). Pure recombinant rabbit IL-1 β (courtesy of Prof. H. Yoshinaga, Kumamoto University, Japan) was employed as the standard in each assay. The lowest IL-1 β concentration detected by the assay was 30 pg/ml. Ex-

perimental meningitis models were also diagnosed by clinical symptoms (lethargy and convulsions) and CSF findings (turbid appearance, increased cell counts and positive bacterial cultures).

2. Cytokines in human CSF

Human samples were analyzed, three times, several weeks apart. Commercially available kits were used to determine the concentrations of human Interleukin-1 β (Research and Diagnostics Systems, Minneapolis, America), Interleukin-6, Interleukin-8 (Toray-Fuji Bionics Inc., Tokyo, Japan) and TNF- α (Genzyme Corporation, Cambridge, America). The determinations of human IL-1 β , IL-6, IL-8 and TNF- α in human CSF were done, using a two-step sandwich method of ELISA. The avidin-biotin amplified method was utilized for the determinations of IL-6 and TNF- α . CSF sample volumes of 50~200 μ l were added to wells of a microtitre plate coated with polyclonal antibodies to IL-1 β and IL-8 and monoclonal antibodies to IL-6 and TNF- α . After incubation for 1~2 h at room temperature to 37°C, the plates were washed, and enzyme-labeled antibodies to IL-1 β and IL-8 and biotinylated IL-6 and TNF- α were added to the wells. After further incubation for 0.5~1 h and washing, horse radish peroxidase (HRP)-conjugate or avidin-HRP conjugate were detected by color development of tetramethylbenzidine substrate and o-phenylenediamine. The ELISA plates were read at 450 nm for IL-1 β , IL-8 and TNF- α and 490 nm for IL-6 on an ELISA ANALYZER ETY-96, (Toyosokki Co., Ltd., Tokyo, Japan) within 20 min after stopping the reaction. The detection limits for IL-6, IL-8 and TNF- α were 10 pg/ml and for IL-1 β was 0.3 pg/ml. Patients with meningitis were diagnosed by the clinical signs, laboratory data: white blood cell count (WBC), erythrocyte sedimentation rate, C-reactive protein in serum and WBC, glucose and protein in

CSF and bacteriological studies (positive bacterial cultures in CSF).

Statistics

The non-parametric chi-square test was used with Yates' correction for statistical analysis.

Results

1. Experimental meningitis model

The concentrations of IL-1 β in CSF from all control rabbits were lower than the detection limit. The mean IL-1 β concentration at 18 h after injection of *H. influenzae* type b was 81.8 ± 2.4 pg/ml (range; 80.0 to 85.0 pg/ml), and that at 24 h was 87.3 ± 15.8 pg/ml (range; 70.0 to 101.0 pg/ml) (Table 1). These values were significantly higher than for control CSF ($p < 0.001$) (Fig. 1).

2. Human meningitis

In CSF samples from 18 children without meningitis, the concentrations of IL-1 β and TNF- α were lower than the detection limit. The mean concentration of IL-6 was 145.1 ± 92.4 pg/ml (range; <10.0 to 285.7 pg/ml), and the mean concentration of IL-8 was 82.1 ± 89.6 pg/ml (range; <10.0 to 233.4 pg/ml). In 26 children with meningitis, the mean concentrations at diagnosis of IL-1 β , IL-6, IL-8 and TNF- α were 56.7 ± 102.1 pg/ml (range; 0.3 to 265.8 pg/ml), 147.2 ± 101.0 pg/ml (range; <10.0 to 333.0 pg/ml), 162.7 ± 91.6 pg/ml (range; <10.0 to 252.8 pg/ml) and 50.4 ± 159.7 pg/ml (range; <10.0

to 670.3 pg/ml), respectively. The TNF- α concentrations from 17 children with aseptic meningitis were lower than the detection limit (Tables 2 and 3). There were no significant differences in the CSF IL-1 β and IL-6 values in CSF of meningitis and control groups, and between groups with septic and aseptic meningitis (Figs 2 and 3). The mean CSF concentrations of IL-8 from the meningitis patients (septic and aseptic combined) were significantly higher than those from control subjects ($p < 0.005$) (Fig. 4). The TNF- α concentrations in aseptic-meningitis and control groups were not detectable (Fig. 5). The CSF samples from 5 patients with septic meningitis and 2 patients with aseptic meningitis were examined twice on admission and after

Fig. 1. Concentrations of IL-1 β in CSF of experimental rabbits.

TABLE 1.
IL-1 β in CSF of experimental rabbits

Hours after inoculation	0	18	24
Mean $\pm$ SD (pg/ml)	<30.0 (10)	81.8 ± 2.4 (4)	87.3 ± 15.8 (3)

() = No. of rabbits

TABLE 2.
IL-1 β , IL-6, IL-8 and TNF- α in CSF during the acute phase

No.	Age (month)	Diagnosis	IL-1 β	IL-6	IL-8	TNF- α	Pathogen
Septic Meningitis (N=9)							
1	12		NT	49.1	248.6	476.4	<i>H. influenzae</i>
2	264		NT	243.1	252.8	162.7	<i>H. influenzae</i>
3	94		NT	NT	241.7	10.0>	unknown
4	60		0.3>	NT	245.5	10.0>	unknown
5	2		NT	NT	241.9	670.7	<i>N. meningitidis</i>
6	5		0.3>	NT	15.2	10.0>	<i>H. influenzae</i>
7	2		265.8	333.0	249.0	10.0>	<i>E. coli</i>
8	2		NT	NT	37.5	10.0>	unknown
9	3		121.0	181.6	247.4	10.0>	<i>S. aureus</i>
Aseptic Meningitis (N=17)							
1	10		NT	NT	208.2	10.0>	
2	4		NT	228.9	130.2	10.0>	
3	48		NT	81.2	237.0	10.0>	
4	60		259.0	36.5	252.2	10.0>	
5	108		0.3>	217.5	20.3	10.0>	
6	8		0.3>	34.9	24.5	10.0>	
7	60		0.3>	247.8	235.2	10.0>	
8	4		0.3>	162.2	11.5	10.0>	
9	24		0.3>	167.4	71.0	10.0>	
10	96		204.2	77.5	177.8	10.0>	
11	84		0.3>	NT	245.9	10.0>	Mumps
12	94		NT	NT	203.4	10.0>	
13	4		0.3>	NT	95.9	10.0>	
14	2		NT	NT	151.2	10.0>	
15	2		0.3>	10.0>	202.1	10.0>	
16	8		0.3>	NT	39.1	10.0>	
17	96		NT	NT	146.0	10.0>	
Control (N=19)							
1	6	Convulsion	0.3>	11.2	10.0>	10.0>	
2	1	Fever	0.3>	25.4	233.4	10.0>	
3	79	FC	0.3>	20.7	230.3	10.0>	
4	12	Measles	NT	46.5	10.0>	10.0>	
5	33	AEC	0.3>	156.7	10.0>	10.0>	
6	8	ICH, Exanthem subitum	NT	175.4	10.0>	10.0>	
7	2	OMA, Colitis	0.3>	94.9	55.5	10.0>	
8	6	FC	0.3>	130.2	39.3	10.0>	
9	144	URI	0.3>	223.0	69.7	10.0>	
10	72	AEC	0.3>	260.1	24.7	10.0>	
11	57	FC, Tonsillitis	NT	224.2	10.0>	10.0>	
12	48	Epilepsy	NT	205.7	181.9	10.0>	
13	48	URI	NT	172.1	152.7	10.0>	
14	84	URI	0.3>	285.7	213.1	10.0>	
15	24	FC	0.3>	NT	10.0>	10.0>	
16	2	Fever	0.3>	NT	10.0>	10.0>	
17	12	FC	0.3>	NT	33.4	10.0>	
18	144	Epilepsy	0.3>	NT	160.4	10.0>	
19	84	AEC	0.3>	NT	162.0	10.0>	<i>Salmonella</i>

N=45 Patients

NOTE. NT: not tested; FC: febrile convulsion; AEC: acute entero colitis; ICH: intra cranial hemorrhage; OMA: acute otitis media; URI: upper respiratory infection

TABLE 3.
Concentrations of cytokines in CSF during the acute phase

Cytokines	Meningitis	Septic meningitis	Aseptic meningitis	Non meningitis
IL-1 β Mean $\pm$ SD (pg/ml)	56.7 $\pm$ 102.1(15)	96.7 $\pm$ 126.3(4)	42.1 $\pm$ 94.5(11)	<0.3 (14)
IL-6 Mean $\pm$ SD (pg/ml)	147.2 $\pm$ 101.0(14)	201.7 $\pm$ 119.2(4)	125.4 $\pm$ 90.3(10)	145.1 $\pm$ 92.4(14)
IL-8 Mean $\pm$ SD (pg/ml)	162.7 $\pm$ 91.6(26)	197.7 $\pm$ 97.4(9)	144.2 $\pm$ 85.6(17)	82.1 $\pm$ 89.6(19)
TNF- α Mean $\pm$ SD (pg/ml)	50.4 $\pm$ 159.7(26)	145.5 $\pm$ 253.1(9)	<10.0(17)	<10.0(19)

() = No. of Patients

Fig. 2. Concentrations of IL-1 β in CSF during the acute phase.

Fig. 4. Concentrations of IL-8 in CSF during the acute phase.

Fig. 3. Concentrations of IL-6 in CSF during the acute phase.

Fig. 5. Concentrations of TNF- α in CSF during the acute phase.

treatment. The interval of CSF examinations ranged from 2 days to 21 days (Table 4). After treatment, the CSF IL-6, IL-8 and TNF- α concentrations decreased

in cases with meningitis (Figs 6, 7 and 8). The CSF concentrations of IL-1 β were lower than the detection limit in these cases.

TABLE 4.
IL-1 β , IL-6, IL-8 and TNF- α in CSF before and after treatment

No.	IL-1 β	IL-6	IL-8	TNF- α	WBC in CSF	Interval	Complication	Pathogen
Septic Meningitis(N=5)								
1	NT 0.3>	49.1 10.0>	248.6 10.0>	476.4 10.0>	3000 816	7 days	nothing	<i>H. influenzae</i>
2	0.3> 0.3>	243.1 134.2	252.8 72.2	162.7 10.0>	10610 NT	21 days	nothing	<i>H. influenzae</i>
3	0.3> 0.3>	NT NT	242.7 241.7	10.0> 10.0>	250 485	2 days	hydrocephalus	unknown
4	0.3> 0.3>	NT NT	245.5 80.52	10.0> 10.0>	5333 747	2 days	nothing	unknown
5	0.3> 0.3>	NT NT	241.9 23.9	670.7 10.0>	62 1390	2 days	death	<i>N. meningitidis</i>
Aseptic Meningitis(N=2)								
1	0.3> 0.3>	NT NT	208.2 178.0	10.0> 10.0>	600 596	2 days	nothing	
2	0.3> 0.3>	NT NT	130.2 10.0>	10.0> 10.0>	76 NT	9 days	nothing	

NOTE. NT: not tested

Fig. 6. Concentrations of IL-6 in CSF before and after treatment.

Fig. 7. Concentrations of IL-8 in CSF before and after treatment.

Fig. 8. Concentrations of TNF- α in CSF before and after treatment.

Discussion

This study was performed to investigate the concentrations of four cytokines (IL-1 β , IL-6, IL-8 and TNF- α) in the CSF from patients with septic or aseptic meningitis as well as from a control group, based upon the demonstration of increased IL-1 β activity in inflamed CSF from experimental models. Changes in individual infectious cytokines have been studied in meningitis patients. However, little data from simultaneous assays of these four cytokines in meningitis patients has been reported.

Larsen et al. (1990) found no significant differences in CSF IL-2 values between septic and aseptic meningitis subjects and Houssiau et al. (1988) reported that IL-6 activities in CSF did not differentiate bacterial meningitis from viral meningitis. In contrast, Perrella et al. (1991) reported that CSF IL-6 values in viral infections or bacterial meningitis were significantly higher than in a control group. In the present study, the data indicated that IL-1 β , IL-6, IL-8 and

TNF- α values in CSF have little value in the diagnosis or discrimination between septic meningitis and aseptic meningitis. IL-8 values in the CSF of both septic and aseptic meningitis patients were, however, significantly higher than those from a control group.

As for the correlation of CSF cytokine levels and the clinical prognosis, McCracken et al. (1989) revealed that peak concentrations of IL-1 β in CSF significantly correlated with the outcome from bacterial meningitis, whereas there was no correlation between the outcome and TNF activities in the CSF. TNF- α levels above the detection limit were found in only 3 septic meningitis patients in the present study, without any detections in both aseptic and control groups; i.e. TNF- α may play a role in pathogenesis of septic meningitis. One of 3 patients with the high TNF- α concentration died.

Perrella et al. (1991) reported that no statistical difference was observed in serum IL-6 levels of meningitis and control groups, whereas there were significantly different levels of the cytokine in the CSF. Assay of the cytokines in the local space where inflammatory reactions take place seems to be more useful for monitoring the clinical outcome and treatment of meningitis, however additional studies are required to elucidate the relationship between CSF cytokine levels and the clinical course in CNS infections.

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